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# Exocytosis of fibrous material from plasmatocytes in *Scutigera coleoptrata* (Chilopoda, Notostigmophora) in relation to wound healing

by

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## ABSTRACT

The plasmatocytes of *Scutigera coleoptrata* were investigated by light and electron microscopy. Plasmatocytes exocytose fibrous structures that develop in their grana. The grana contain an electron-dense area in their centre, from which the fibrous material detaches. The grana transform themselves to vacuoles. The tubules may be released from the vacuoles of the plasmatocytes, either individually or in compact clusters into the hemolymph, where they probably support coagulation. Hemolymph coagulation observed in *S. coleoptrata* is remarkably strong. This special coagulation behaviour correlates functionally with the special leg-autotomy-capabilities of these animals. *S. coleoptrata* lives predominantly in dry habitats, where fast wound healing would appear to have a selective advantage in securing survival.

## INTRODUCTION

The Notostigmophora occupy a distinct position within the Chilopoda. They are the adelphotaxon to the pleurostigmophoran Chilopoda. Besides specialised tracheal lungs (Hilken 1994 1997 1998) and a unique maxillary organ (Haase 1885), they show hemolymph particularities (Sundara Rajulu 1969 1974). *Thereuopoda longicornis* (Newport, 1845) was one of the first representatives of the Chilopoda of which the hemolymph composition was investigated (‘*Scutigera longicornis*’, Sundara Rajulu 1974) and where the presence of the respiratory pigment hemocyanin was proved (Sundara Rajulu 1969; Mangum 1985; Gebauer 1999). However, the knowledge about the hemolymph and the hemocyte repertoire in chilopods is still limited. Few species have been investigated in this regard so far: *Ethmostigmus p. spinosus* (Newport, 1845), Scolopendromorpha (Sundara Rajulu 1970); *Scolopendra morsitans* (Linné, 1758), Scolopendromorpha (Sundara Rajulu 1971), *Lithobius forficatus* (Linné, 1758), Lithobiomorpha (Nevermann *et al.*™ 1991); *Scutigera coleoptrata* (Linné, 1758), Notostigmophora (Hilken *et al.*, in press).

In *S. coleoptrata* four types of hemocytes were identified: prohemocytes, plasmatocytes, granulocytes, and spherulocytes. They differ in shape, size, ultrastructural characteristics and their staining reaction (Hilken *et al.*, in press). Only few studies focused on functional aspects of hemocytes in Myriapoda based on electron microscopical techniques (Nevermann *et al.* 1996; Nevermann & Xylander 1996; Hilken *et al.*, in press).

The habitats of *S. coleoptrata* are hot and semi-arid areas. In Europe they are therefore most common in the Mediterranean area. They are known as house centipedes because of their habit of looking for humidity and shelter in houses, but they also occur under rocks and in well-holes. Because of their special leg-autotomy-capability, these animals are good subjects for investigating aspects of wound healing, which is of essential importance for animals living in dry habitats. The present study describes fibrous, tubular structures exocytosed from plasmatocytes of *S. coleoptrata*. Their origin and their role in the wound healing process are discussed.

## METHODS

Two specimens of *S. coleoptrata* were collected by the first author in July 1996 around Banyuls-sur-Mer, France at 42°29' N, 03°7' E. The specimens were kept in glass boxes (15 cm x 8 cm x 15 cm) filled with 1 cm garden soil, and two pieces of moistened (tap water) blotting paper (15 cm x 15 cm each). The animals were fed with wingless *Drosophila melanogaster* (Linné, 1758) and *Musca domestica* (Linné, 1758). Specimens were killed with $CO_2$, cut into several parts and preserved as described below.

Transmission electron microscopy: Single segments of *S. coleoptrata* were fixed in 2.5 % glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4, 3 hours, 4 °C) and repeatedly washed in the same buffer. They were postfixed with 1% $OsO_4$ (1 h) in the same buffer and dehydrated in acetone. Propylene oxide was used as an intermediate for embedding the objects in Epon. Ultrathin cross sections were stained with uranyl acetate and lead citrate (Watson 1958) and examined under a ZEISS EM 902 A electron microscope.

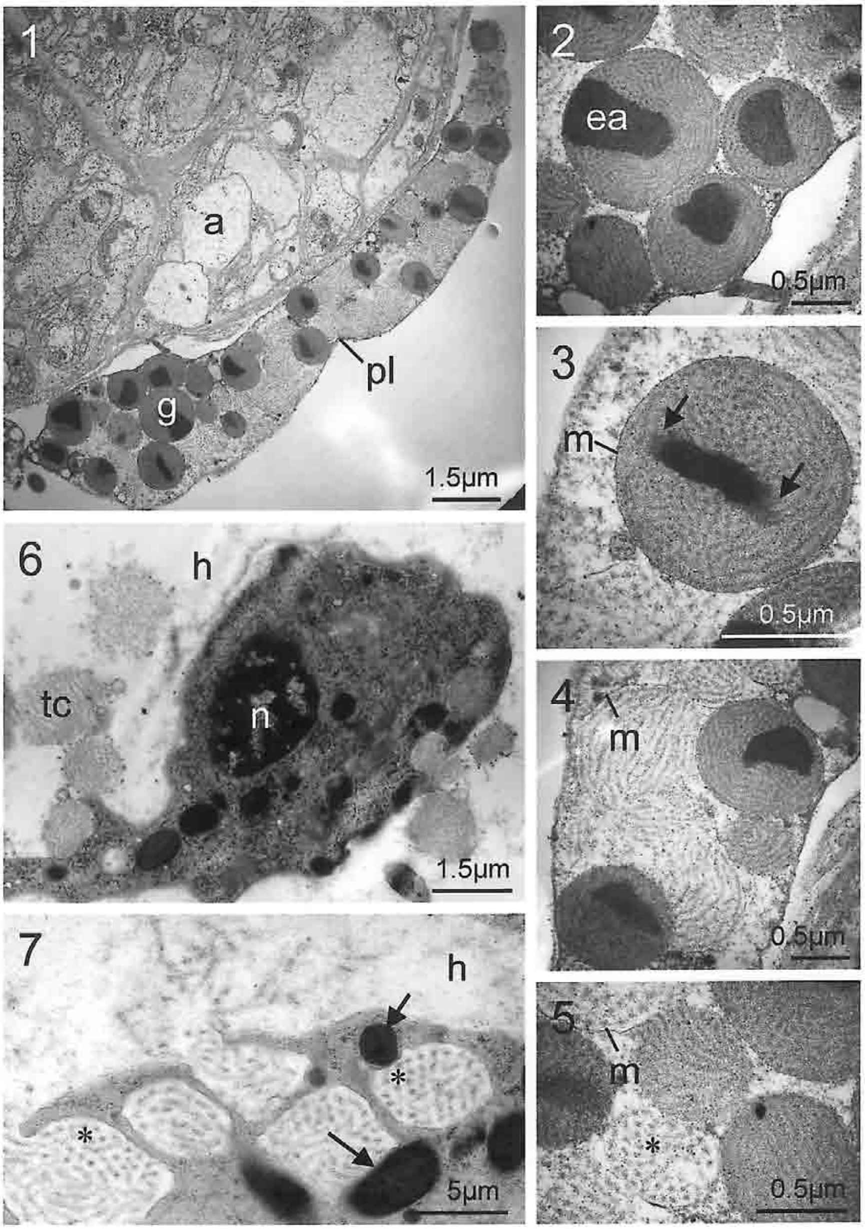
## RESULTS

Plasmatocytes represent the predominant hemocyte type in the hemolymph of *S. coleoptrata*. The length of these cells varies between 4.3 and 11.6 µm. The nuclei are mainly oval to irregular and are located in the centre or at the margin of the cells (Fig. 6). The cells contain large vacuoles, mitochondria, dictyosomes, multivesicular bodies and numerous (mainly electron dense) grana 0.1–1.0 µm in diameter. The number of these grana varies (Figs 6, 7). They are oval or spherical shaped. Some of these grana show an array of parallel structures in longitudinal sections (Fig. 7, short arrow) that appears tubular in transverse sections (Fig. 7, long arrow).

These grana are surrounded by a distinct membrane (Figs 3, 4) and are filled with numerous fibrous tubules. A distinctively darker electron-dense area is located in the centre of the grana (Figs 1–4) and shows the same array of fibrous tubules. Figure 3 (arrows) shows a marginal detachment of tubuli from that centre. The tubuli surrounding the central area are less compactly arranged compared to the above mentioned type of grana (Fig. 7, arrows). Adjacent to the tubuli-filled grana, areas of tubuli with distinctively larger, but regular, interspaces are found (Fig. 4). Here, the surrounding membrane is found only in fragments (Figs 4, 5, *m*).

Besides their presence in grana, most tubular structures are located in vacuoles (Figs 6, 7). The tubuli measure 0.4 µm in length. The central cavities of the tubules have a diameter of 15 nm (Fig. 7, asterisk), the outside diameter is 45 nm. Some plasmatocytes show marginal vacuoles opening to the hemolymph and emitting tubular material of loose (Fig. 7) or relatively compact (Fig. 6) structure.

The tubules are more compactly arranged intracellularly than after their exocytosis into the hemolymph (Fig. 7).



Figs 1–7. Plasmatocytes of *Scutigera coleoptrata*.1. Plasmatocyte (*pl*) with several grana (*g*) containing fibrous material. The hemocyte is attached to an axon (*a*). 2. Numerous grana, containing tubular fibrous substances and electron dense areas (*ea*). 3. Details of a granulum with an electron dense area. The arrows show a marginal detachment of tubuli from that centre; grana membrane (*m*). 4. Grana and areas where grana membranes (*m*) are dissolved; note that the fibrous tubuli remain ordered. 5. Cross sectioned fibrous tubuli (asterisk), (*m*) membrane. 6. Plasmatocyte, releasing tubuli in clusters (*tc*) into the hemolymph (*h*), nucleus (n). 7. Plasmatocyte, releasing tubuli singly into hemolymph. Tubuli in longitudinal section (long arrow), tubuli in cross section (short arrow). Asterisk indicates cross-sectioned tubuli.

## DISCUSSION

The plasmatocytes of *S. coleoptrata* contain numerous electron-dense grana and some vacuoles filled with tubular material. It has been assumed that these tubules originate from the electron-dense grana (Hilken *et al.*, in press). The present study provides strong evidence that the tubules containing vacuoles have their origin in the electron-dense grana. The transitional, spherical stages shown here are characterised by the detachment of tubules from a condensed, electron-dense core.

After 'maturation' of the tubules within the grana, the grana appear to transform themselves to vacuoles (Fig. 7). Since the fibrous material was also observed in the cytoplasm, it is assumed that the membrane of the grana rupture (Figs 4, 5) under the process of exocytosis. The tubules may be released either individually or in compact clusters from the vacuoles, into the hemolymph. In the latter case, the tubules are presumably interconnected.

In the cytoplasm, filaments were observed in the areas that are involved in releasing tubuli from vacuoles into the hemolymph. These filaments stabilise the exocytosis areas (Hilken *et al.*, in press).

The special mode of exocytosing clusters of fibrous material as described in the present study has been observed only in *S. coleoptrata* (Hilken *et al.*, in press), but very similar tubuli within grana were also described by Hagopian (1971) in 'premelanosomes' of freshly moulted cockroach nymphs (*Leucophaea maderae* Fabricius, 1775), in hemocytes of *Melolontha melolontha* (Devauchelle, 1971), and in plasmatocytes and granulocytes of the chilopods *L. forficatus* and *Scolopendra cingulata* Latreille, 1829. In the latter the grana swell and form large vacuoles before clouds of fibrous material are ejected (Nevermann *et al.* 1991; Nevermann 1996). Furthermore, a fibrin-like coagulation of this fibrous material is reported for *L. forficatus* and *S. cingulata* (Nevermann 1996).

In the Xiphosuran *Tachypleus tridentatus*, for which the coagulation system has been very well investigated, tubule containing grana and vacuoles appear to be very similar to those of insects and chilopods (Hagopian 1971; Jakobsen & Suhr-Jessen 1990; Toh *et al.* 1991; Nevermann *et al.* 1991; Hilken *et al.*, this study). The grana of *Tachypleus tridentatus* were shown to contain clotting enzymes and coagulogen (Toh *et al.* 1991). It is likely that the fibrous material, observed here in *S. coleoptrata*, shares the same characteristics and function.

The hemolymph coagulation in *S. coleoptrata* is remarkably stronger than in other centipedes and cannot be inhibited even by anti-coagulation buffer (Hilken *et al.*, in press). This special coagulation behaviour correlates functionally with the special leg-autotomy-capabilities of these animals. *Scutigera coleoptrata* lives predominantly in dry habitats, where fast wound healing would appear to have selective advantage in securing surviving.

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